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The White Knight hypothesis, or does the environment limit innovations?

Andreas Wagner[a,b,c]

[a]University of Zurich, Institute of Evolutionary Biology and Environmental Studies, Zurich, Switzerland,

[b]Swiss Institute of Bioinformatics, Lausanne, Switzerland,

[c]Santa Fe Institute, Santa Fe, New Mexico, USA

Abstract.

Organisms often harbor latent traits that are by-products of other adaptations. Such latent traits are not themselves adaptive, but they can become adaptive in the right environment. Here I discuss several examples of such traits. Their abundance suggests that environmental change rather than new mutations might often limit the origin of evolutionary adaptations and innovations. This is important, because environments can change much faster than new mutations arise. I introduce a conceptual model that distinguishes between mutation-limited and environment-limited trait origins, and suggest how experiments could help discriminate between them. Wherever latent traits are plentiful, ecology rather than genetics might determine how fast new adaptations originate, and thus how fast adaptive Darwinian evolution proceeds.

Prolific inventors. When the White Knight rescues Alice, the heroine of Lewis Carroll's story *Through the Looking Glass*, he tells her of his many inventions. They include a box whose lid is on the bottom to keep the rain out, and a device for trapping mice should they appear on his horse's back.

> "You see," he went on after a pause, "it's as well to be provided for *everything*. That's the reason the horse has all those anklets round his feet."
> "But what are they for?" Alice asked in a tone of great curiosity.
> "To guard against the bites of sharks," the Knight replied. "It's an invention of my own." [1]

The White Knight might be a hopeless inventor, given that horses rarely encounter sharks. However, even biological evolution, an enormously successful "inventor", has produced organismal traits that might be useless in most environments. For example, bacteria isolated from 5,000 year old permafrost cores can not only survive on semi-synthetic antibiotics, such as amikacin, they can use them as sole sources of carbon [2]. Likewise, microbial isolates from pristine soil with minimal or no exposure to human civilization can thrive on synthetic antibiotics such as ciprofloxacin [3], which likely do not occur in nature. Even laboratory evolution experiments can create traits that are not adaptive in their environment of origin. For example, a laboratory evolution experiment aimed at evolving a *Pseudomonas putida* strain that metabolizes xylose efficiently, created an evolved strain that thrived equally well on arabinose [4]. (Neither did the environment contain arabinose, nor was the ancestral strain able to metabolize it.)

One might call traits like these "White-Knight" traits. They are not themselves adaptations, because they exist as by-products of other traits. Instead, in the terminology of Stephen Jay Gould, they are potential exaptations [5], because they might become useful in environments different from where they arose. They require us to distinguish between the origin of a heritable trait – usually through mutations – and its eventual "success" in an environment where the benefit the trait provides helps natural selection spread it through a population. Recent evidence suggests that such traits are widespread [6-12], which requires us to rethink a

fundamental question in evolutionary biology: What limits the origin of beneficial new traits, including the qualitatively new traits often referred to as evolutionary innovations?

Common genetic wisdom has it that the origin of evolutionary innovations is primarily limited by mutations, and experimental evidence shows that this is indeed often the case [13-15]. For example, in a population of *E.coli* evolving in the laboratory on glucose minimal medium supplemented with citrate, it can take 30,000 generations before mutations arise that allow cells to metabolize citrate [15]. More generally, evolving populations frequently experience episodic evolution, where long bouts of stasis alternate with brief bursts of adaptive evolution that are caused by new mutations [13, 14, 16-18]. However, if latent, potentially exaptive traits are abundant, then environmental change could be just as important as mutation in enabling the appearance of new and beneficial traits.

Here I address the question how one could quantitatively distinguish between these scenarios, which I will refer to as M-limited and E-limited adaptive evolution. I begin with recent work on latent traits which suggests that E-limitation might be more frequent than we appreciate. Then I introduce a conceptual model to quantify M-limitation and E-limitation of adaptive evolution. Finally, I discuss how experiments might help distinguish between M-limited and E-limited innovation.

Some sources of White-Knight traits. One frequent source of White Knight traits is promiscuous enzymes. Such enzymes catalyze one main biochemical reaction, usually at a high specific activity, and one or more "side reactions" at lower activity. Promiscuous enzymes occur in animals, plants, and prokaryotes. They include not only metabolic enzymes, but also enzymes that help synthesize plant and pathogen defense molecules, and that help degrade environmental toxins [6, 7, 19-25]. What is more, promiscuous enzymes are very widespread. For example, in *E. coli* alone, 37 percent of metabolic enzymes are promiscuous [21].

Promiscuity is a consequence of protein biophysics [6]. It is thus often not adaptive in itself, but it can have important adaptive consequences. For example, a combination of computational and experimental analyses showed that the metabolic reactions added to the *E.coli* metabolism through known promiscuous *E.coli* enzymes can help *E.coli* survive in 19 additional environments, i.e., on 19 new sources of chemical elements and energy. Moreover,

these reactions can increase *E.coli's* growth in 31 new environments [24]. Promiscuity is likely also behind many instances where bacteria isolated from pristine environments can survive on unusual molecules, such as man-made antibiotics [2, 3]. For example, β-lactamases, proteins that confer resistance against naturally occurring β-lactam antibiotics, are well-known promiscuous proteins [6, 26].

Even without promiscuity, complex biochemical systems can harbor non-adaptive abilities that emerge as by-products of adaptations. A case in point is a recent study that took advantage of the ability to predict an organism's viability on specific nutrients merely from information on its metabolic genotype, and in good agreement with experimental data, [27, 28]. The study showed that organisms viable on glucose as a sole carbon source would usually also happen to be viable on multiple other carbon sources, simply as a by-product of the complexity of their biochemical reaction network [12]. These alternative carbon sources can occur in different metabolic pathways than the primary source.

While latent traits are easiest to characterize in microbes, E-limitation can also occur in other organisms and their traits. Consider invasive species, such as the spotted knapweed (*Centaurea maculosa*) or the Monterey pine (*Pinus radiata*), which are enormously successful in new habitats, even though their native habitats might be geographically restricted [29, 30]. *P. radiata*, for example, is native to the central coast of California and Mexico, but has invaded three other continents, where it occurs in habitats as different as grasslands and Eucalyptus forests [30]. The reasons for such invasive success are debated and varied, and include release from natural enemies such as specialist herbivores [31-35]. The chemical defenses of plants against herbivores are prime candidates for traits that might exhibit latency, and not only because plants produce a bewildering array of some 200,000 different defense chemicals [36]. Most importantly, these chemicals are usually synthesized by promiscuous enzymes or enzyme families that create complex product mixtures [6, 37, 38]. Only some of these products might be effective against native enemies, whereas others could be fortuitously useful in a newly invaded habitat. Conversely, insect herbivores like the black swallowtail (*Papilio polyxenes*) can adapt to plant toxins by diversifying already promiscuous detoxification enzymes, such as cytochrome P450 monooxygenases [37]. The promiscuous activities of such enzymes might help herbivores survive in novel environments.

The distinction between mutational and environmental limitation applies equally well to non-metabolic traits, for example those involving changes in gene regulation. Regulatory adaptations involve genes that form regulatory circuits. These genes' expression patterns guide myriad processes from cell physiology to embryonic development. Not surprisingly then, novel gene regulation and novel gene expression are also involved in many new adaptations. These range from modifications of existing traits, such as the size of butterfly eyespots or of defensive spines in stickleback fish, to entire new body plans [39-41]. Regulatory circuits and their target genes have primary expression patterns necessary for their biological function, but they often also show secondary, accessory expression patterns that might not have any adaptive significance [9, 42-44]. For example, the extracellular metalloprotease encoded by the Drosophila gene *Neprylisin-1* has acquired novel expression in the optic lobe of the developing nervous system of *Drosophila santomea* in the last few million years [10]. The human *ENO2* gene encodes an enolase with a conserved role in the mouse nervous system, but it is also expressed in the human uterus without any known biological function [43]. While it is difficult to prove with certainty that such latent gene expression patterns are non-adaptive, the rate of gene expression evolution in general suggests that latent gene expression frequently emerges non-adaptively through genetic drift [9, 43, 44]. Moreover, computational models of transcriptional regulation circuits show that latent expression patterns are frequent and inevitable by-products of a circuit's normal function [11]. Latent expression patterns illustrate that the emergence of beneficial regulatory traits need not be M-limited. It can be E-limited wherever the right environment can turn a latent gene expression pattern into a beneficial trait.

All these potential examples of White-Knight traits do not imply that mutations are unnecessary for evolutionary adaptation. To the contrary, when a White-Knight trait turns beneficial, mutations might be essential to improve the trait's phenotypic expression, and thus an organism's fitness. This is especially important for promiscuous enzymes, which often show weak activity on a new substrate. Whenever this activity becomes fitness-determining, activity-increasing mutations will also increase fitness, and become essential for an innovation's persistence and refinement [19, 45].

A conceptual model to quantify E-limited and M-limited adaptive evolution. The following modeling framework uses the language of resource use, but its concepts apply to many other kinds of traits, such as the chemical defense traits of plants. Consider a universe

of *U* possible resources (sources of energy and chemical elements) that an organism could in principle use to sustain life. And consider an environment that contains some subset R_E of these resources, as well as an organism that is viable on some (possibly different) subset R_O of these resources. In terms of ecological niche theory [46], R_O is analogous to the breadth of an organism's fundamental resource niche, that is, the number of resources on which it could survive in the absence of interactions with other organisms. I note that such biotic interactions can alter the breadth of the actual or "realized" niche [47]. For example, one organism's waste products can provide a resource that can facilitate another organism's growth through cross-feeding. Conversely, the presence of a resource-consuming competitor can render one or more resources unavailable. Because of my focus on latent, i.e., potential traits, I will focus on the fundamental rather than the realized niche. In addition, although the model could be extended to explicitly consider fitness, which will depend on the amount of any one available resource, I confine myself to the qualitative criterion of viability for simplicity and clarity.

Assume that an organism can survive on any resource in R_O when it is the only resource, just like *E.coli* can survive on glucose as the sole source of carbon and energy. If no resource on which the organism is viable occurs in the environment (i.e., if $R_O \cap R_E = \emptyset$) then the organism will perish. Viability requires that at least one resource in the environment can also sustain the organism's life, i.e., that $|R_O \cap R_E| \geq 1$. If $R_O = R_E$, all resources in the environment can be used by the organism and vice versa. In consequence, no mutation that allows an organism to survive on an additional resource could be an adaptation in this environment. However, if the environment harbors all resources usable by the organism and also additional resources that the organism cannot use (i.e., $R_O \subset R_E$), then DNA mutations are required to use those resources (which lie in $R_E \setminus R_O$, i.e., the subset of R_E that lies outside of R_O). In this scenario, innovation is strictly mutation-limited. Conversely, if the organism can use all resources in the environment and also additional resources (i.e., $R_E \subset R_O$), then the organism harbors latent innovations that will not become of adaptive value unless the environment changes. In this case, innovation is strictly environment-limited. In a third, mixed scenario, the environment harbors some resources that the organism cannot use, and the organism can use some resources that are not available in the environment (i.e., neither $R_O \subset R_E$ nor $R_E \subset R_O$ hold). In this case, innovation will be mutation-limited for the first class of resources (those in $R_E \setminus R_O$) and environment-limited for the second class of resources (those in $R_O \setminus E$). In other words, E- and M-limitation are not mutually exclusive (Figure 1, Key Figure).

For a more quantitative analysis, assume that different environmental resources are independently and equally likely to occur in any one environment, such that the environment has a probability p_E of containing any one resource. The greater the parameter p_E, the more resource-rich the environment is. Conversely, assume that an organism has a probability p_O of being viable on any one resource, and that viability on different resources is statistically independent across resources. The parameter p_O encapsulates the extent to which an organism is versatile in its resource use – the greater p_O is, the more resources the organism can survive on. (I deliberately avoid the term generalist here, because it implies adaptation, whereas latent viability on some resources need not be an adaptation.) Although these assumptions – especially independence – will often not be met in practice, the model can easily accommodate more general assumptions.

When is innovation most strongly limited by the environment? This question is addressed in Figure 2a, which shows a contour plot of the expected number of resources that are not available in the environment, but that can be used by an organism viable in this environment, for varying degrees of environmental richness ($0 \leq p_E \leq 1$, x-axis) and organismal versatility ($0 \leq p_O \leq 1$, y-axis). Environmental change will be most important for adaptation in a highly versatile organism living in a resource poor environment (upper left corner). The organism's ability to use these resources is limited only by the environment itself, such that environmental change would be necessary to use them. This is not the case for a specialist organism in a resource rich environment (lower right corner), for a specialist in a resource poor environment (lower left corner), and for a versatile organism in a resource-rich-environment (upper right corner). The white region corresponds to combinations of specialization and resource-richness that are least conducive to innovation by environmental change. The darker the red shading, the greater is the percentage of resources for which environmental change is essential for adaptation. I note that a genetically variable population of organisms might be more versatile than any one of its member organisms [48, 49], such that genetic variation might increase p_O, R_O, and thus the importance of E-limited innovation.

Figure 2b addresses the converse question: When is innovation most strongly limited by mutation. In other words, when is the expected percentage of resources in *U* that is available but cannot be used by a viable organism largest? The Figure shows a contour plot of this expected percentage for all possible values of environmental richness ($0 \leq p_E \leq 1$) and

organismal versatility ($0 \leq p_O \leq 1$). Mutations are most necessary for innovations when a resource specialist lives in a resource-rich environment (lower right corner). Conversely, a versatile organism living in a resource-poor environment (upper left corner), will have the fewest opportunities for adaptation through mutation. The same holds for a specialist in a resource-poor environment (lower left corner), and a versatile organism in a resource-rich environment (upper right corner). The white region is least conducive to innovation by mutation. The darker the blue shading, the greater is the percentage of resources on which adaptive evolution through mutation is possible.

Figure 2c, finally, superimposes the information from Figure 2a and Figure 2b, to indicate the exaptation or innovation potential of an organism in a given environment, regardless of the cause of the innovation. The darker the shade of red (blue), the greater is this potential through environmental change (mutation). The potential for exaptation through environmental change is greatest for a versatile organism in a resource-poor environment. Conversely, the potential for adaptation through mutation is greatest for a specialist in a resource-rich environment.

Versatile organisms are able to sustain their lives on multiple different resources. Among them are *Salmonella typhimurium*, which can use 25 different sources of nitrogen [50], *Desulfobulbus rhabdoformis* [51], which can use eight different electron donors and four different electron acceptors, and *E.coli*, which can survive on at least 54 carbon sources [52]. However, these numbers are minute compared to the thousands of different molecules that could in principle serve as sources of energy and chemical elements. Thus, even highly versatile organisms can probably use only a small fraction of the resource universe, and any one environment might contain only a small fraction of these resources. This means that most organisms and environments in which innovation takes place will be located in the lower left quadrant of Figure 2c. There, as environmental richness increases along the horizontal axis, so does the potential of mutation to create new adaptations. And as organismal versatility increases along the vertical axis, so does the potential of environmental change to create new exaptations.

E-limited innovation might be widespread from paleontology to technology. The traditional paleontological view on the origin of new organismal forms emphasizes the importance of *adaptive radiations*. During such a radiation, novel, adaptive forms originate

through changes intrinsic to an organism, and help organisms expand into new ecological niches that already exist in the environment [53, 54]. However, the existence of *macroevolutionary lags* suggests that there is more to macroevolution than adaptive radiations. Such lags refer to a delay between the origin of a major clade and its eventual widespread success. One example involves grasses (Poaceae), which already existed in the Oligocene some 30 million years ago. However, they remained of minor ecological importance, and became a widespread and ecologically dominant plant family only millions of years later, in the Miocene, when the environment became drier and created the conditions that allowed grasslands to expand [55]. Another example involves lucinid bivalves, a family of marine clams, which originated more than 400 million years ago in the Silurian, but did not become widespread and diverse until some 200 million years later, in the late Cretaceaous. Responsible for their belated success are mangroves and seagrasses, which had spread by the Cretaceous, and created anaerobic sulfur-rich environments on the seafloor. These environments – an example of niche construction [56] – helped sulfur-oxidizing endosymbiotic bacteria of lucinids to synthesize organic carbon molecules for their bivalve host [57]. The success of grasses and lucinid bivalves are clear examples of E-limitation. More generally, macroevolutionary lags show that extrinsic, environmental changes can be critical to the success of already existing, latent innovations [54, 55, 57, 58].

Similar phenomena exist in the human realm, where the process of technological innovation has many parallels to biological evolution [59, 60]. Economists and historians of technology sometimes distinguish mere inventions, technological novelties that need not be useful (think: White Knight) from innovations, which are successful in a company or the marketplace. Importantly, extrinsic factors can be critical for an invention's success. For example, even though wheels were well-known in pre-Columbian Mesoamerica, they did not serve transportation but are largely found on small toys or ritual objects. The likely reasons include a terrain unfriendly to wheeled transport, and an absence of large draught animals to pull heavy carts [61]. Incandescent lighting (lightbulbs) could not become truly successful until humans mastered large-scale electric power generation and distribution. Edison was instrumental for creating the environment in which it could thrive, perhaps more so than for developing better lightbulbs [62].

Experimental validation and concluding remarks. All these examples lend anecdotal support to what I call the "White-Knight" hypothesis: The environment is more important

than intrinsic factors, such as mutations, in limiting the origin of new and beneficial traits. Unfortunately, in technology, paleontology, and for many complex traits of extant species, it might be impossible to provide more than anecdotal support for this hypothesis. But in some cases, quantifying M-limitation and E-limitation can become experimentally feasible. This holds especially for culturable microbes and their physiological phenotypes, where high-throughput phenotyping platforms, such as BIOLOG phenotyping microarrays [63, 64] help measure resource utilization on hundreds of different sources of chemical elements (including antibiotics). These resources represent the most common members of the resource universe U, and additional phenotypes can be easily determined [65]. Phenotyping microarrays have been used successfully to study the metabolic potential of microbial communities sampled from different environments, such as soil and freshwater [65-68], but they can also be applied to individual culturable prokaryotic isolates When applied, for example, to carbon sources, they can provide information on the number r_O of carbon sources an organism can utilize. To find out how many of these resources (r_E) are available in the organism's environment is a bigger challenge, but even that challenge can be met. For example, one study analyzed resource utilization from rhizosphere microbial samples of different grasslands on r_E=52 carbon sources known to be prominent in root exudates [65]. Such data can not only provide information on the fraction of these carbon sources on which an organism is viable ($|R_O \cap R_E|/|U|$), but the ratios p_E=r_E/|U| and p_O=r_O/|U| can help locate an organism on a diagram like that of Figure 2c. An approach like this could help quantify whether metabolic innovation in a given organism and environment is primarily M-limited or E-limited, and it could help address multiple empirical questions about the incidence of E-limited innovation. The White-Knight hypothesis might be true for only some species and traits, but wherever it is true, evolutionary innovation becomes primarily a problem for ecology rather than genetics.

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Figure Caption

Figure 1, Key Figure: The relationship between latent (White-Knight) traits, E(nvironmentally)-limitated, and M(utationally)-limited evolutionary innovation. Both the upper and the lower Venn diagram show the intersection of two shapes. The left shape (red and purple) represents the set of resources R_O an organism can use, regardless of whether these resources are available in a given environment (latent traits). The right shape (blue and purple) represents the set of resources R_E that are available in the environment, regardless of whether the organism can use them. The intersection (purple, $R_O \cap R_E$) corresponds to adaptive resource-use traits. The red and blue areas correspond to latent traits and unused resources, respectively. Innovation is strongly E-limited (upper Venn diagram) if there are many more latent resource-use traits than unused resources, such that a changing environment where new resources become available can render these latent traits beneficial. Innovation is strongly M-limited (lower Venn diagram) if there are many more unused resources than latent traits, such that mutations are necessary to take advantage of these resources. The language used here is that of resource use, but the concepts apply to many other traits, for example a plant's spectrum of herbivore-defense molecules, or a gene regulatory circuit's adaptive and latent gene expression patterns.

Figure 2: A conceptual model for the relationship between mutationally (M) and environmentally (E) limited evolutionary innovation. All three panels show the extent of environmental richness (p_E) on the horizontal axis, where a specific value of p_E indicates the fraction of all possible resources *U* that are available in an environment. They show the extent to which an organism is versatile (p_O) on the vertical axis, that is, the fraction of all resources that the organism could potentially use. Denote as r_E and r_O random variables for the number of resources that are available in the environment and that are usable by the organism, respectively. By the assumption of independence for resource occurrence and viability, these random variables are binomially distributed with means Up_E and Up_O. Denote as r_{EO} the total number of resources that occur in the environment *and* that are usable by the organism. **a)** Color intensity indicates the expected percentage of resources that an organism can use and that are not in the environment, conditioned on the organism's viability, which is denoted as $\mathbf{E}(r_O - r_{EO}|V)$ (see below for calculation). **b)** Color intensity indicates the expected percentage of resources that an environment harbors, and that an organism cannot use, conditioned on the organism's viability, which is denoted as $\mathbf{E}(r_E - r_{EO}|V)$. **c)** The sum of

the expected values for the upper panels, i.e., $\mathbf{E}(r_O - r_{EO}|V) + \mathbf{E}(r_E - r_{EO}|V)$, indicating the total number of resources for which innovation is possible, regardless of whether it requires mutation or environmental change. To calculate these expectations, I note that one can compute the probability of finding a given set of values $(r_{EO,}r_E, r_O)$ as $P(r_{EO,}r_E, r_O) = \binom{U}{r_E} p_E^{r_E}(1-p_E)^{U-r_E}\binom{r_E}{r_{EO}}\binom{U-r_E}{r_O-r_{EO}} p_O^{r_O}(1-p_O)^{U-r_O}$, if $\max(r_E + r_O - U, 0) \leq r_{EO} \leq \min(r_E, r_O)$. If r_{EO} does not fall within this interval, then $P(r_{EO,}r_E, r_O) = 0$. The preceding inequality for r_{EO} describes the range of admissible values for r_{EO}, which lie between (i) the minimally possible overlap r_{EO} that two sets of resources must have, and (ii) their maximally possible overlap. (For example, r_{EO} cannot be larger than the smaller of the two sets R_E and R_O, hence $r_{EO} \leq \min(r_E, r_O)$.) From these relationships, one can calculate the probability that an organism is viable and that the environment harbors an excess of *i* resources that the organism cannot use as $P(r_E - r_{EO} = i) = \sum_{r_E=i+1}^{U} \sum_{r_{EO}=r_E-i}^{U-i} P(r_E - i_{,}r_E, r_O)$. Using Bayes' theorem, one can then calculate the expected number of these resources, conditioned on the viability of an organism as $\mathbf{E}(r_E - r_{EO}|V) = (\frac{1}{P_V}) \sum_{i=0}^{U} i\, P(r_E - r_{EO} = i)$, where $p_V = 1 - \sum_{r_E=0}^{U} \sum_{r_O=0}^{U-r_E} P(0_{,}r_E, r_O)$ is the probability that an organism is viable, i.e., that it can use at least one resource available in the environment. Analogously, one can calculate the probability that an organism is viable and that it can use exactly i resources that do not exist in the environment as $P(r_O - r_{EO} = i) = \sum_{r_O=i+1}^{U} \sum_{r_E=r_O-i}^{U-i} P(r_O - i_{,}r_E, r_O)$. The corresponding expected number of resources is given by $\mathbf{E}(r_O - r_{EO}|V) = (\frac{1}{P_V}) \sum_{i=0}^{U} i\, P(r_O - r_{EO} = i)$. The model assumes different resources to occur independently, and to be utilizable independently, a condition that might not often be met in nature, for example because promiscuous enzymes tend to use chemically related substrates [6]. But while relaxing this assumption would change the shape of the contour lines, it would not affect the most important qualitative relationships, for example that innovation is environment-limited for versatile organisms in resource-poor environments.

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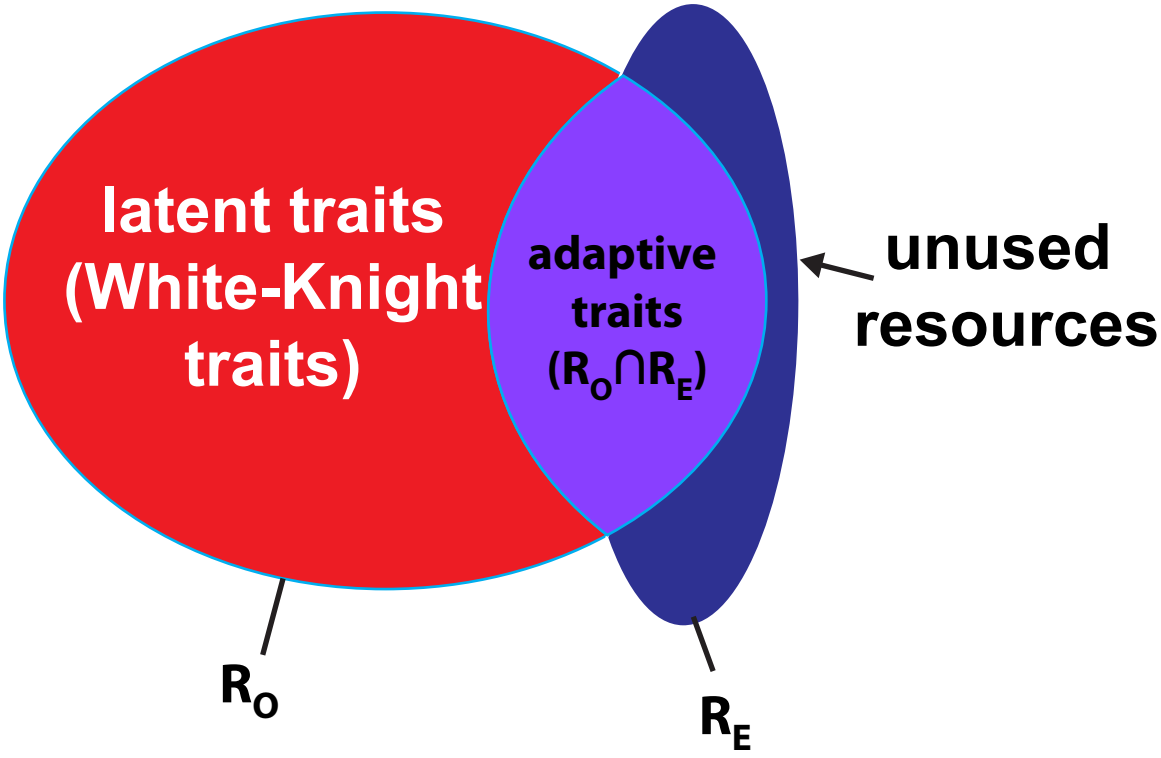
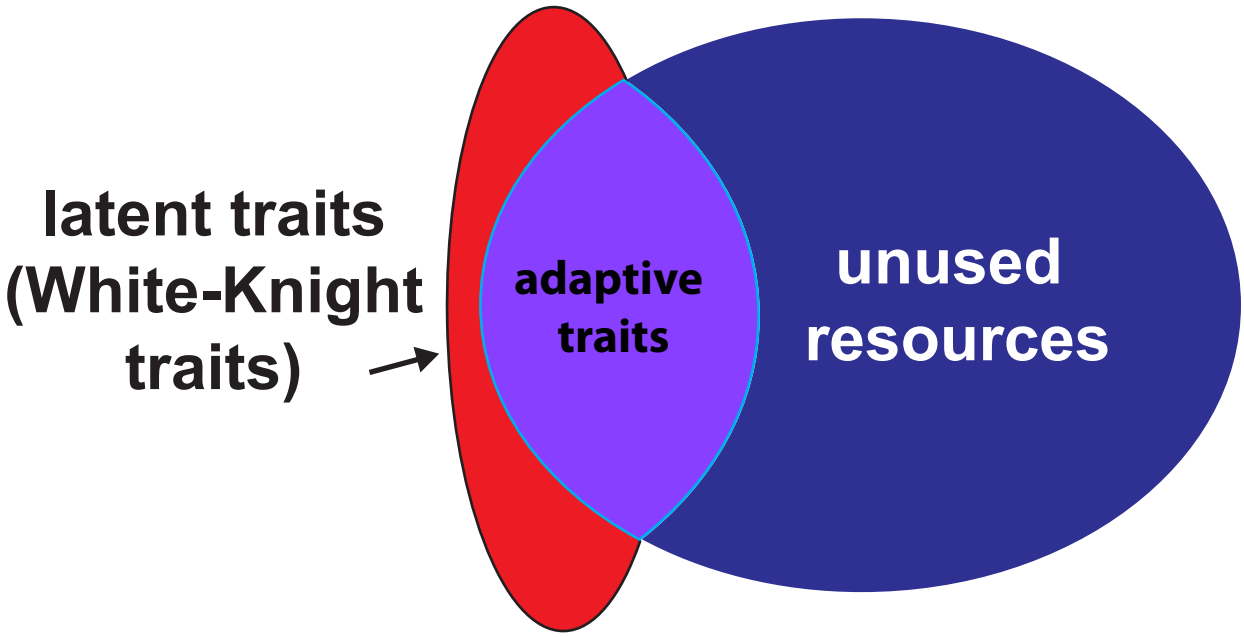
Strong E(nvironmental)-limitation



Strong M(utation)-limitation



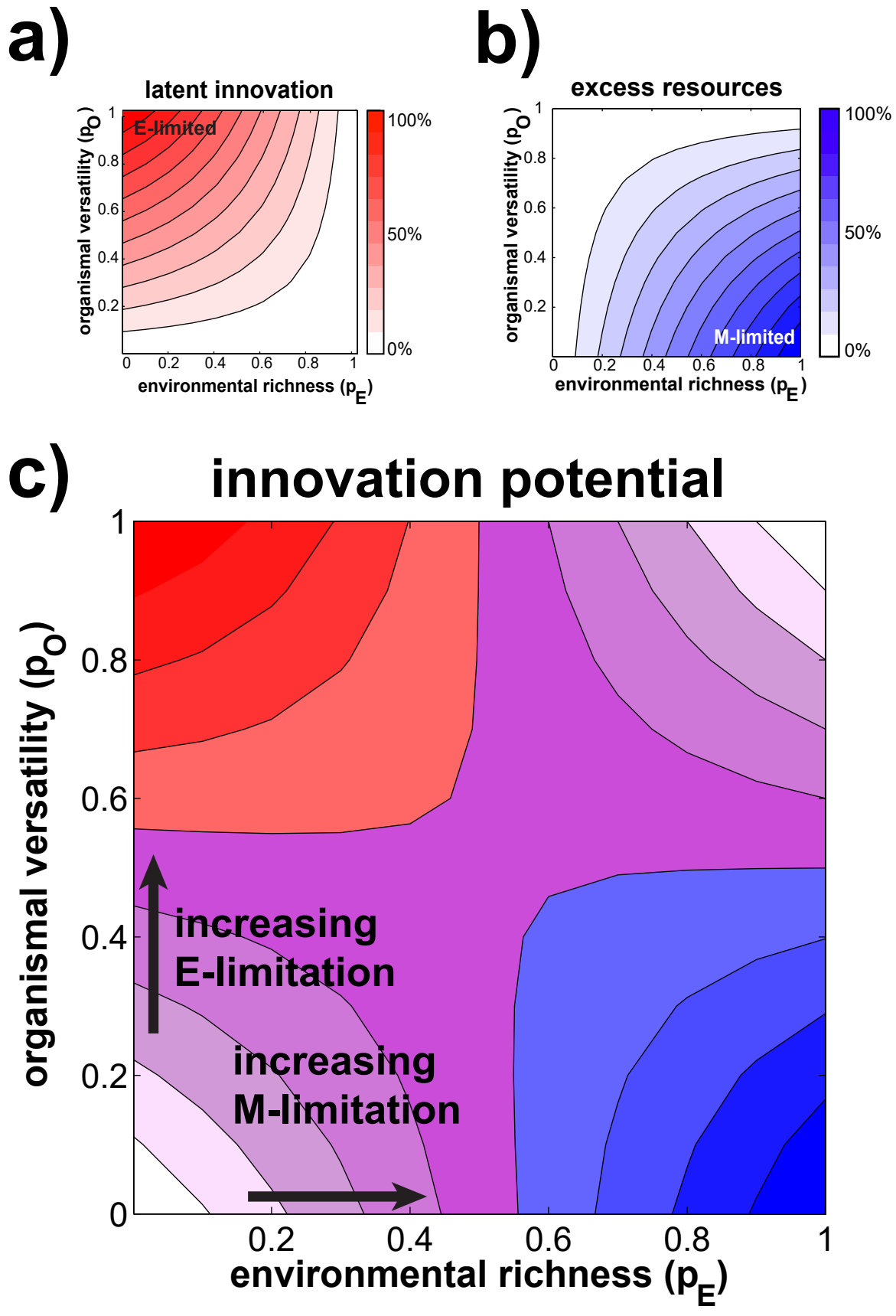

a)
latent innovation
E-limited
organismal versatility (p_O)
environmental richness (p_E)
100%
50%
0%
b)
excess resources
M-limited
organismal versatility (p_O)
environmental richness (p_E)
100%
50%
0%
c)
innovation potential
increasing E-limitation
increasing M-limitation
organismal versatility (p_O)
environmental richness (p_E)

Figure 1